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No. PCT/DK2004/000001
Owner: Symphogen A/S
'Manufacturing of polyclonal antibodies'

Dear Sirs,

In response to the written opinion from the International Searching Authority dated 28 September 2004 and in advance of the international preliminary examination, we hereby provide our comments to the issues raised in said written opinion.

As an initial remark, we wish to note that the "Basis of the opinion" in item 4 ("additional comments") indicates that a copy has not been furnished of the earlier application whose priority has been claimed. However, as appear from the enclosed Form PCT/IB/304 dated 29 April 2004, all 3 priority documents have been furnished to the International Bureau of WIPO. We therefore request that the Examiner considers the question of validity of the priority claims in the present application.

In the claims

We have deleted claims 41-47. The remaining claims have been maintained in unamended form.

In the following we will comment on the objections on an item-by-item basis:

Item V-1, novelty of claims 41-47

The deletion of these claims should render this objection moot.

Item V-2, inventive step of claims 1-40

The Examiner states that independent claims 1, 32 and 37 lack an inventive step over D1 when combined with D2.

We do not agree.

The present invention pertains to a method for generating a collection of cells producing a polyclonal protein, where all members of the polyclonal protein exhibit binding affinity for the same antigen, cf. the wording of claim 1 in step a: "...wherein each of said vectors comprises one single copy of a distinct nucleic acid sequence encoding a distinct member of a polyclonal protein comprising distinct members ***that bind a particular antigen***..." (our emphasis).

The invention further pertains to a method for producing a polyclonal protein (claim 21), a manufacturing cell line that produces the polyclonal protein (claim 32) and to a library of vectors useful in the methods (claim 37).

In other words, the present invention in general relates to production of complex mixtures of proteins where *all* proteins share the same feature of *binding a desired antigen* and are *produced in the same batch*. One important feature here is to maintain a balanced production of each of the species in the polyclonal protein, so that the relative amounts of each of the species is not changed dramatically during e.g. fermentation.

Hence, an important technical feature of the present invention is that *all* expression vectors used for production of cells in claim 1 encode proteins that bind the desired target. The same technical feature appears in independent claims 21, 32 and 37.

In contrast, the teachings of D1 and D2 both relate to the preparation of libraries that are *useful for subsequent screening* – that means that the focus of both references is the production of *highly diverse* libraries that can include a maximum number of different species showing *variable binding properties* (if showing any binding properties at all), from which proteins with the desired binding properties can be selected. No particulars concerning over-time stability of production of such diverse libraries are reported in any of the references.

In particular, D1 relates to the preparation of yeast expression vectors encoding diverse libraries of two polypeptide subunits where both subunits vary independently in the library (D1, page 36, lines 9-15). These expression vectors can be prepared by means of homologous recombination in yeast (D1, pages 54-67). The expression vectors and transformed cells can be subsequently used for screening in various systems, e.g. yeast two-hybrid or one-hybrid systems, for members of the libraries that bind a particular target molecule (D1, pages 67-98 and Figures 4 to 6).

According to D1, it is important that the libraries are extremely diverse, cf. D1, page 27, lines 3-6, and page 28, lines 7-19.

From pages 98 and onwards, D1 does in fact discuss production of those members of the libraries that bind a particular target molecule. However, nowhere in this section in D1 or anywhere else is it contemplated or discussed that *several* such "positive" library vectors should be introduced into cells in order to prepare a *polyclonal* manufacturing cell line. What seems to

be contemplated by D1 is simply that the identified library vectors are transferred to traditional monoclonal expression systems where they are separately expressed and harvested.

D2 similarly relates to methods of expressing libraries of expression vectors with a view to use these in screening so as to identify those that encode proteins that bind target molecules of choice (cf. the paragraph bridging pages 42 and 43).

In contrast to D1, D2 relates to eukaryotic non-yeast cell expression systems and vectors, but otherwise the teachings in D1 and D2 are very similar. For instance, D2 also relates to use of homologous recombination (D2, page 19 lines 12-30) and a "one gene – one cell" approach, both factors that facilitate subsequent identification of members of the library that bind a specific target molecule.

The Examiner has indicated in item V.2(v) that the teachings in D2 would indicate to the skilled person that the system taught therein has a minimized clonal variation – we can agree that this perhaps is true when it comes to the variations in the numbers and location of introduced vectors in each cell, but D2 does not in any way indicate that a polyclonal population of transformed cells will necessarily be stable over time with respect to the relative production rate of individual members of the library. In fact, the passage at page 16 cited by the examiner uses the term "creation of *isogenic* cell lines" (our emphasis), which indicates that the collection of cells generated by incorporation of the variant nucleic acids are subsequently selected to obtain cell lines which are isogenic with respect to one particular variant. Hence, the polyclonal population of transformed cells is not maintained as such, for production purposes. This is supported by the examples of D2, where a geneticin selection is performed prior to screening the individual clones (isogenic cell lines) for expression of a protein which binds a desired target.

Hence, when considering D1 and D2 these disclosures may superficially resemble the presently claimed invention because of the use of certain technical solutions, but there are some very important differences.

The most important is, that none of the two references teach that all expression vectors must encode proteins that bind a desired target, and this basically resides in the fact that the *objects* of the present invention and of the disclosures in D1 and D2 are very different.

Since D1 and D2 both aim at producing *diverse* protein libraries for screening purposes, there is absolutely no focus on the stability of the relative expression of single members of the protein libraries. Thus, when producing a library for screening purposes, it is important for the skilled person that as many members of the library are produced as possible, but there is no need for ensuring that members in the library are not changing their rate of expression over time – this is so, because a very diverse library of vectors inevitably will express members that can be identified via their binding and

subsequently can be used for a particular purpose. Further, the period of cultivation is very short in a screening assay, whereas for production purposes the cultivation period will extend over many weeks.

This difference is not trivial, because the conception of the present invention is based on the surprising finding that it is indeed *possible* to prepare polyclonal manufacturing cell lines that in a stable manner ensure uniform production over time of the individual species comprising the polyclonal protein. Without this knowledge, it is not possible to arrive at the presently claimed invention from neither D1 nor D2. D1 and D2 merely teach that it is possible to prepare protein libraries that are useful for screening purposes but not that it is possible to prepare polyclonal mixtures of proteins that bind a desired target *and* are suitable for e.g. pharmaceutical preparations (which require a minimal batch-to-batch variation).

If the skilled person considered D1 as closest prior art for any purpose, it would be for the production of libraries useful for screening, and here we do not disagree with the Examiner that he would turn to D2 in order to improve and expand the technology disclosed in D1.

However, nowhere in neither D1 nor D2 can we find any indications that would prompt the skilled person to prepare a collection of cells by means of the method of present claim 1, *i.e.* by limiting the introduction of vectors in a host cell line to those vectors that encode proteins that bind a particular antigen.

In fact, the closest prior art to the present invention is in our opinion disclosures pertaining to preparation of mixtures of proteins where each member of the mixtures bind the same target molecule, *i.e.* disclosures such as those discussed in the paragraph bridging pages 16 and 17 of the present application. However, there is nothing in neither D1 nor D2 that could provide the skilled person with the knowledge that a collection of cells comprising a population of variant nucleic acids would exhibit a stability over time that would render feasible the production over time of a stable mixture of antibodies as a single batch.

More simply phrased, since none of the disclosures in D1 and D2 teach the use of vectors that encode proteins binding a desired target (antigen), it is not possible to arrive at the presently claimed invention by combining these two references.

It is therefore our opinion that the presently presented claims meet the requirements of Art. 33(2) and (3) PCT.

However, should the International Preliminary Examining Authority be of a differing opinion, it is requested that a second written opinion be issued to allow the applicant to expand on the views set forth above.

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Please confirm safe receipt of this letter and its enclosures by means of the enclosed EPO Form 1037.

Yours sincerely
Inspicos A/S

Peter Koefoed
European Patent Attorney

Copy of Form PCT/IB/304 dated 29 April 2004
Amended claims
EPO Form 1037

10/540227

JC17 Rec'd PCT/PTO 21 JUN 2005

CLAIMS

1. A method for generating a collection of cells suitable as a recombinant polyclonal manufacturing cell line, said method comprising:

a) providing a library of vectors comprising a population of variant nucleic acid sequences, wherein each of said vectors comprises 1) one single copy of a distinct nucleic acid sequence encoding a distinct member of a polyclonal protein comprising distinct members that bind a particular antigen and 2) one or more recombinase recognition sequences;

b) introducing said library of vectors into a host cell line, wherein the genome of each individual cell of said host cell line comprises recombinase recognition sequences, matching those of the vector, at a single specific site in its genome;

c) ensuring the presence in said cells of one or more recombinases so that the variant nucleic acid sequences of step (a) are integrated site-specifically in the cells of the host cell line, where said one or more recombinases is/are either i) expressed by said cells into which said nucleic acid sequence is introduced; ii) operatively encoded by the vectors of step a; iii) provided through expression from a second vector; or iv) provided to the cell as a protein; and

d) selecting cells comprising an integrated copy from said library of variant nucleic acid sequences.

2. The method according to claim 1, wherein the polyclonal protein is not naturally associated with said collection of cells.

3. The method according to claim 1 or 2, wherein said polyclonal protein is a polyclonal antibody or antibody fragment.

4. The method according to claim 1 or 2, wherein said polyclonal protein is a polyclonal T cell receptor or T cell receptor fragment.

5. The method according to any one of the preceding claims, wherein said library of vectors is introduced into said host cell line by bulk transfection of a collection of said host cells with said library of vectors.

6. The method according to any one of claims 1-4, wherein said library of vectors is introduced into said host cell line by semi-bulk transfection of aliquots of said host cells with fractions comprising 5 to 50 individual vectors of said library of vectors, and said cells are pooled to form a collection of cells suitable as a recombinant polyclonal manufacturing cell line prior or subsequent to the selection of step (d).

7. The method according to any one of claims 1-4, wherein said library of vectors for site-specific integration is introduced into said host cell line by transfecting said host cells separately with individual members of said library of vectors, and said cells are pooled to form a collection of cells suitable as a recombinant polyclonal manufacturing cell line prior or subsequent to the selection of step (d).

8. The method according to any one of the preceding claims, wherein the population of variant nucleic acids in step (a) are isolated or identified by the aid of a screening procedure that enables identification and/or isolation of nucleic acids that encode protein which bind said particular antigen.

9. The method according to claim 8, wherein the screening procedure includes a biopanning step and/or an immunodetection assay.

10. The method according to claim 8 or 9, wherein said screening procedure is selected from the group consisting of phage display, ribosome display, DNA display, RNA-peptide display, covalent display, bacterial surface display, yeast surface display, eukaryotic virus display, ELISA and ELISPOT.

11. The method according to any one of the preceding claims, wherein said library of variant nucleic acid sequences comprises at least 3 variant nucleic acid sequences.

12. The method according to any one of the preceding claims, wherein individual members of said library of variant nucleic acid sequences are integrated in a single predefined genomic locus of individual cells in said collection of cells, said locus being capable of mediating high-level expression of each member of said recombinant polyclonal protein.

13. The method according to any one of the preceding claims, wherein each distinct nucleic acid sequence comprises a pair of gene segments that encode a member of a polyclonal protein comprised of two different polypeptide chains.

14. The method according to claim 13, wherein said pair of gene segments comprise an antibody heavy chain variable region encoding sequence and an antibody light chain variable region encoding sequence.

15. The method according to claim 13, wherein said pair of gene segments comprise a T cell receptor alpha chain variable region encoding sequence and a T cell receptor beta chain variable region encoding sequence.

16. The method according to claim 13, wherein said pair of gene segments comprise a T cell receptor gamma chain variable region encoding sequence and a T cell receptor delta chain variable region encoding sequence.

17. The method according to any one of the preceding claims, wherein said library of variant nucleic acid sequences comprises a naturally occurring diversity located within the variant nucleic acid sequences.

18. The method according to claim 17, wherein the naturally occurring diversity is located in CDR regions present in said variant nucleic acid sequences.

19. The method according to any one of the preceding claims, wherein said collection of cells is derived from a mammalian cell line or cell type.

20. The method according to claim 19, wherein said mammalian cell line is selected from the group consisting of Chinese hamster ovary (CHO) cells, COS cells, BHK cells, YB2/0, NIH 3T3, myeloma cells, fibroblasts, HeLa, HEK 293, PER.C6, and cell lines derived thereof.

21. A method for the manufacture of a polyclonal protein, wherein said polyclonal protein comprises distinct members that bind a particular antigen, said method comprising:

a) providing a collection of cells comprising a library of variant nucleic acid sequences, where each of said nucleic acid sequences encode a distinct member of said polyclonal protein and where each of said nucleic acid sequences are integrated at the same, single site of the genome of each individual cell in said collection of cells;

b) culturing said collection of cells under conditions facilitating expression of said polyclonal protein; and

c) recovering said expressed polyclonal protein from the cell culture cells or cell culture supernatant.

22. The method according to claims 21, wherein the collection of cells in step (a) is generated according to the method of any one of claims 1-20.

23. The method according to claim 21 or 22, wherein the polyclonal protein is not naturally associated with said collection of cells.

24. The method according to any one of claims 21-23, wherein the library of variant nucleic acids in step (a) are isolated or identified in an earlier step by the aid of a screening procedure that enables identification and/or isolation of nucleic acids that encode protein which bind said particular antigen.

25. The method according to claim 24, wherein the screening procedure includes a biopanning step and/or an immunodetection assay.

26. The method according to claim 24 or 25, wherein said screening procedure is selected from the group consisting of phage display, ribosome display, DNA display, RNA-peptide display, covalent display, bacterial surface display, yeast surface display, eukaryotic virus display, ELISA, and ELISPOT.

27. The method according to any one of claims 21-26, wherein said polyclonal protein is a polyclonal antibody or antibody fragment.

28. The method according to any one of claims 21-26, wherein said polyclonal protein is a polyclonal T cell receptor or T cell receptor fragment.

29. The method according to any one of claims 21-28, wherein the relative expression levels of the variant nucleic acid sequences are monitored.

30. The method according to claim 29, wherein said expression levels are monitored at mRNA level and/or protein level.

31. The method according to claim 29 or 30, wherein the culturing in step (b) is terminated at the latest when the relative expression levels are outside a predetermined range.

32. A recombinant polyclonal manufacturing cell line comprising a collection of cells transfected with a library of variant nucleic acid sequences, wherein each cell in the collection is transfected with and capable of expressing one member of the library, which encodes a distinct member of a polyclonal protein that binds a particular antigen and which is located at the same single site in the genome of individual cells in said collection, wherein said nucleic acid sequence is not naturally associated with said cell in the collection.

33. The recombinant polyclonal manufacturing cell line according to claim 32, wherein said library of variant nucleic acid sequences encodes a polyclonal antibody or antibody fragment having a naturally occurring diversity among the individual members of said polyclonal antibody or antibody fragments.

34. The recombinant polyclonal manufacturing cell line according to claim 32, wherein said library of variant nucleic acid sequences encodes a polyclonal T cell receptor or T cell receptor

fragment having a naturally occurring diversity among the individual members of said polyclonal T cell receptor or T-cell receptor fragment.

35. The recombinant polyclonal manufacturing cell line according to any one of claims 32-34, wherein said collection of cells is derived from a mammalian cell line or cell type.

36. The recombinant polyclonal manufacturing cell line according to claim 35, wherein said mammalian cell line is selected from the group consisting of Chinese hamster ovary (CHO) cells, COS cells, BHK cells, YB2/0, NIH 3T3, myeloma cells, fibroblasts, HeLa, HEK 293, PER.C6, and derivative cell lines thereof.

37. A library of vectors for site-specific integration comprising a population of naturally occurring variant nucleic acid sequences, wherein each of said vectors comprises 1) one copy of a distinct nucleic acid sequence encoding a distinct member of a polyclonal protein that binds a particular antigen and 2) one or more recombinase recognition sequences.

38. The library according claim 37, wherein said population of naturally occurring variant nucleic acid sequences encode a polyclonal antibody or antibody fragment.

39. The library according claim 37, wherein said population of naturally occurring variant nucleic acid sequences encode a polyclonal T cell receptor T cell receptor fragment.

40. The library according to any one of claims 37-39, wherein each member of said library of vectors further comprises a recombinase encoding nucleic acid sequence.